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The Oxidation of Acetylmethylcarbinol to Diacetyl in Butter Cultures

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CONCLUSIONS

1. The addition of purified acetylmethylcarbinol to sterile skimmilk, adjusted to an acidity and a temperature satisfactory for the rapid production of the carbinol when the citric acid-fermenting streptococci are present, did not result in the formation of appreciable amounts of diacetyl in 48 to 72 hours. The results were the same when carbon dioxide, hydrogen, nitrogen, or oxygen was bubbled through the milk as when no gas was used.

2. The production of diacetyl in acidified skimmilk cultures of the citric acid-fermenting streptococci was definitely influenced by bubbling various gases through the freshly acidified cultures. Oxygen regularly gave a higher yield of diacetyl than the control through which no gas was bubbled, while carbon dioxide, hydrogen or nitrogen gave lower yields. With all of the gases there was commonly a greater production of diacetyl, as well as acetylmethylcarbinol plus diacetyl, when the cultures were acidified with a mixture of citric and sulfuric acids than when acidified with sulfuric acid alone.

3. Various gases had the same general effect on the production of diacetyl in butter cultures as in pure cultures of the citric acid-fermenting streptococci, but the actual quantities of diacetyl formed appeared to be smaller with the butter cultures than with the pure cultures. When oxygen was bubbled through butter cultures prepared with various amounts of added citric acid, the yields of both diacetyl and acetylmethylcarbinol plus diacetyl were roughly proportional to the amount of citric acid added.

4. When acetylmethylcarbinol was added to milk and the milk inoculated with *S. lactis*, analyses after incubation showed no evidence of an oxidation of the carbinol to diacetyl.

5. The data obtained indicate that the oxidation of acetylmethylcarbinol to diacetyl in a butter culture is due to the activity of the citric acid-fermenting streptococci rather than to a direct chemical oxidation.

The Oxidation of Acetylmethylcarbinol to Diacetyl in Butter Cultures[1]

By M. B. Michaelian and B. W. Hammer

Diacetyl, acetylmethylcarbinol, and 2,3-butylene glycol constitute a series of compounds that is of special importance in butter cultures and, presumably, in butter manufactured with the use of culture. A satisfactory butter culture commonly contains relatively large quantities of acetylmethylcarbinol that are formed through the activity of the citric acid-fermenting streptococci normally present in the culture (2). Small portions of this acetylmethylcarbinol appear to be oxidized to diacetyl which is important from the standpoint of the aroma of the culture. Some of the acetylmethylcarbinol is also reduced to 2,3-butylene glycol through the action of the citric acid-fermenting streptococci (1). The reduction is much slower in a butter culture than in a pure culture of one of the streptococci to which acetylmethylcarbinol has been added, and this relatively slow reduction is probably due to the inhibitory effect of the acid in the butter culture on these streptococci.

From the standpoint of the aroma of a butter culture, the oxidation of acetylmethylcarbinol to diacetyl is especially important since diacetyl, in the proper concentration, has an odor suggestive of fine butter, while highly purified acetylmethylcarbinol is odorless. Accordingly, an attempt was made to determine whether this oxidation is a direct chemical action or whether it is brought about through the activity of the citric acid-fermenting streptococci. The general procedure followed was to study the effect of carbon dioxide, hydrogen, nitrogen, and oxygen on the production of diacetyl (a) in acidified sterile milk to which acetylmethylcarbinol had been added, (b) in acidified cultures of the citric acid-fermenting streptococci, and (c) in butter cultures.

METHODS

Diacetyl was determined as nickel dimethylglyoximate (3), using either a 200 or 400-gm. sample for analysis; carbon dioxide or nitrogen was bubbled through the system for some time before the distillation was begun, in order to remove oxygen which might produce diacetyl from acetylmethylcarbinol during the process. Acetylmethylcarbinol was also determined as nickel dimethylglyoximate by oxidizing the carbinol in a 200-gm. sample to diacetyl with ferric chloride and distilling it as diacetyl (3).

[1] Project 127 of the Iowa Agricultural Experiment Station.

The pH determinations were made electrometrically, using quinhydrone.

When gas was to be bubbled through milk, water, or a culture, the material was placed in a bottle fitted with a stopper carrying two glass tubes. One of the tubes, which was used as the inlet tube, led nearly to the bottom of the bottle while the other, or outlet tube, terminated a very short distance below the stopper. Gas from a cylinder was bubbled through the material rather rapidly for about 10 minutes; although the material was agitated by the current of gas, it was occasionally shaken. When the treatment was complete, clamps were placed on the rubber connections leading to the glass tubes. A few lots of milk foamed considerably but as a rule no difficulty was encountered.

Commercial acetylmethylcarbinol was purified by washing with cold anhydrous ether, as suggested by Stahly and Werkman (5).

EXPERIMENTAL

The attempts to oxidize acetylmethylcarbinol to diacetyl in milk, without the action of bacteria, were carried out as follows: A quantity of sterile skimmilk was acidified to a pH satisfactory for the rapid production of acetylmethylcarbinol when the citric acid-fermenting streptococci are present. Usually a mixture of citric (0.15 percent) and sulfuric (0.28 to 0.30 percent) acids was employed to give a pH of 3.8 or 3.9 because under these conditions the citric acid-fermenting organisms produce the carbinol in relatively large amounts; in one case lactic acid was used to give about the pH found in a ripened butter culture. A solution of acetylmethylcarbinol was then added and various gases bubbled through different portions of the milk. After 48 to 72 hours at 21° C. determinations of acetylmethylcarbinol plus diacetyl and of diacetyl were made. Table 1 presents representative data.

In each trial the quantities of acetylmethylcarbinol plus diacetyl found in the various portions of milk were essentially the same. When commercial or partially purified acetylmethylcarbinol was added to the milk, diacetyl was found on distillation, but the quantities in the different portions of milk used in a trial showed no regular variations and bubbling carbon dioxide, hydrogen, nitrogen or oxygen through the milk did not significantly influence the amount. With the addition of purified acetylmethylcarbinol to the milk, appreciable quantities of diacetyl were not obtained with any of the gases.

The results show that when acetylmethylcarbinol was added to milk there was no appreciable formation of diacetyl in 48

TABLE 1. EFFECT OF VARIOUS GASES ON ACETYLMETHYLCARBINOL IN ACIDIFIED, STERILE SKIMMILK.

Acetylmethylcarbinol and acid added to sterile skimmilk; various gases bubbled through different portions and containers sealed; amc* + ac_2 and ac_2 determined after holding 48 or 72 hours at 21°C.

amc used	Acid used	pH at once	Hours held	Gas bubbled through milk	gm. Ni dimethylglyoxamate ⊃ to	
					amc + ac_2 per 200 gm.	ac_2 per 200 gm.
Commercial	0.15% citric .29% sulfuric	3.8	48	none	0.1241	0.0080
				CO_2	.1271	.0081
				H_2	.1274	.00815
				O_2	.1247	.0070
Commercial	0.15% citric .30% sulfuric	3.8	48	none	.1222	.0059
				CO_2	.1195	.0058
				H_2	.1167	.00685
				O_2	.1176	.00675
Partially purified	0.15% citric .30% sulfuric	3.9	48	none	.1129	.00385
				CO_2	.1163	.00285
				H_2	.1177	.00335
				N_2	.1079	.0025
				O_2	.1110	.0032
Purified	0.15% citric .30% sulfuric	3.9	48	none	.1386	none
				CO_2	.1437	none
				H_2	.1406	none
				N_2	.1343	none
				O_2	.1364	none
Purified	0.15% citric .28% sulfuric	3.9	48	none	.0485	none
				CO_2	.0492	none
				H_2	.0469	none
				N_2	.0491	none
				O_2	.0445	none
Purified	0.15% citric .30% sulfuric	3.8	72	none	.1989	none
				CO_2	.1953	none
				H_2	.1872	none
				N_2	.1896	none
				O_2	.1822	none
Purified	0.70% lactic	4.4	72	none	.1486	none
				CO_2	.1470	none
				H_2	.1475	none
				N_2	.1477	none
				O_2	.1458	none

*amc = acetylmethylcarbinol.
ac_2 = diacetyl.

to 72 hours at an acidity and a temperature satisfactory for the rapid production of the carbinol when the citric acid-fermenting streptococci are present.

Attempts to oxidize acetylmethylcarbinol to diacetyl in a short time were also carried out in water. Acetylmethylcarbinol was added to sterile water, various gases were bubbled through different portions and the acetylmethylcarbinol plus diacetyl and the diacetyl determined after 48 hours at 21° C. When purified acetylmethylcarbinol was used, diacetyl was not found with carbon dioxide, hydrogen, nitrogen, oxygen, or in the controls.

In some cases small quantities of oxidizing reagents were added to the water solutions (without the use of a gas) in an attempt to oxidize the acetylmethylcarbinol. Hydrogen peroxide and potassium permanganate did not produce diacetyl in 48 hours at 21° C. Potassium dichromate yielded a trace of diacetyl but as much diacetyl was obtained when the mixture was distilled at once as when it was distilled after 48 hours which suggests that the diacetyl was produced during the distillation process rather than by the effect of the potassium dichromate at 21° C.

The effect of various gases on the production of diacetyl by the citric acid-fermenting organisms in milk was studied as follows: Lots of sterile skimmilk were inoculated with the organism to be used and incubated at 21° C. After acidifying with citric (0.15 percent) and sulfuric (usually 0.30 percent) acids to a pH satisfactory for the rapid production of acetylmethylcarbinol, various gases were bubbled through the different lots. The usual determinations were made following 48 hours of incubation at 21° C. The data obtained are given in table 2.

In each trial there was some variation in the yields of acetylmethylcarbinol plus diacetyl with the various gases and in certain of the trials the variations were very large. None of the gases regularly gave either the highest or lowest production of the carbinol. There were relatively large variations in the yields of diacetyl and the largest yield was always obtained when oxygen had been bubbled through the culture while the second largest yield was always obtained with the culture exposed to the air. In a number of instances there was a comparatively low yield of acetylmethylcarbinol plus diacetyl with oxygen or exposure to air and at the same time the yield of diacetyl was relatively high. There was no regular variation in the yields of diacetyl with carbon dioxide, hydrogen, and nitrogen.

The data indicate that the production of diacetyl by the citric acid-fermenting streptococci was definitely modified by bubbling various gases through the cultures and that oxygen gave the largest yield of diacetyl, air gave the next largest yield, while carbon dioxide, hydrogen, and nitrogen gave relatively low yields.

Additional results on the effect of various gases on the production of diacetyl by the citric acid-fermenting organisms were obtained by inoculating lots of sterile skimmilk with one of the organisms, incubating 24 hours at 21° C., acidifying some lots with sulfuric acid (0.37 or 0.38 percent) and other lots

TABLE 2. EFFECT OF VARIOUS GASES ON ACIDIFIED CULTURES OF THE CITRIC ACID-FERMENTING STREPTOCOCCI.

Sterile skimmilk inoculated and held at 21°C.; 0.15% citric acid and enough sulfuric acid (usually 0.30%) to give the desired pH then added; various gases bubbled through different portions and containers sealed; amc + ac_2 and ac_2 determined after holding 48 hours at 21°C.

Organism used	Hours incub. before adding acids	pH after adding acid	Gas bubbled through milk	gm. Ni dimethylglyoxamate ⪥ to	
				amc + ac_2 per 200 gm.	ac_2 per 200 gm.
9	24	3.4*	none	0.0649	0.0046
			CO_2	.0669	.0009
			H_2	.0830	.0006
			N_2	.0869	.0012
			O_2	.0420	.0076
34	24	3.9	none	.1153	.00475
			CO_2	.1056	.0029
			H_2	.1094	.0022
			O_2	.0943	.00825
49	24	3.9	none	.0901	.00375
			CO_2	.0840	.00295
			H_2	.0912	.00315
			N_2	.0953	.0033
			O_2	.0738	.00505
146	24	3.8	none	.0822	.0062
			CO_2	.0876	.0036
			H_2	.0850	.00375
			O_2	.0869	.01075
146	24	3.9	none	.0771	.0089
			CO_2	.0957	.0077
			H_2	.0975	.00795
			N_2	.1010	.00735
			O_2	.0881	.0125
29	40		none	.0653	.0094
			CO_2	.1194	.0013
			O_2	.0771	.0139
37	40		none	.0431	.0041
			CO_2	.0673	.0013
			O_2	.0981	.0122
37	40		none	.0916	.0043
			CO_2	.0684	.0019
			O_2	.0790	.0082

*0.40% sulfuric used to get low pH.

with citric (0.15 percent) and sulfuric (0.31 or 0.32 percent) acids, bubbling various gases through the different lots and then making the usual analyses after 48 hours at 21° C. Table 3 gives the data obtained with two organisms.

With each organism the addition of citric acid gave the expected increase in the production of acetylmethylcarbinol plus diacetyl. Either with or without the addition of citric acid there was some variation in the yields of acetylmethylcarbinol plus diacetyl in the presence of the various gases, but these were not large except in the case of one of the organisms without added citric acid. There were relatively large variations

TABLE 3. EFFECT OF VARIOUS GASES ON CULTURES OF THE CITRIC ACID-FERMENTING STREPTOCOCCI ACIDIFIED WITH SULFURIC ACID OR WITH CITRIC AND SULFURIC ACIDS.

Sterile skimmilk inoculated and held 24 hours at 21°C.; sulfuric acid added to some portions and citric and sulfuric acids to others; various gases bubbled through different portions and containers sealed; amc +ac_2 and ac_2 determined after holding 48 hours at 21°C.

Organism used	Acidity adjusted with	pH after adding acid	Gas bubbled through milk	gm. Ni dimethylglyoxamate ⊂ to	
				amc +ac_2 per 200 gm.	ac_2 per 200 gm.
D2	0.38% sulfuric	3.9	none	0.0364	trace
			CO_2	.0364	none
			H_2	.0354	trace
			O_2	.0392	0.0045
	0.15% citric .32% sulfuric	3.9	none	.0912	.0067
			CO_2	.1026	trace
			H_2	.1081	trace
			O_2	.0971	.0115
29	0.37% sulfuric	3.9	none	.0248	.0048
			CO_2	.0190	.0007
			H_2	.0175	trace
			O_2	.0369	.0093
	0.15% citric .31% sulfuric	3.9	none	.0779	.0090
			CO_2	.0756	.0032
			H_2	.0743	.0034
			O_2	.0802	.0181

in the yields of diacetyl. The largest yield was regularly obtained when oxygen had been bubbled through the culture and in three of the four cases exposure to air gave a strikingly larger yield than carbon dioxide or hydrogen. Commonly there was more diacetyl present in the cultures containing a relatively large amount of acetylmethylcarbinol plus diacetyl, as the result of the addition of citric acid, than in the cultures containing a comparatively small amount of the carbinol. The differences were particularly striking with oxygen and exposure to air.

The effect of bubbling various gases through cultures of one of the citric acid-fermenting organisms on the production of diacetyl when citric acid alone was used to acidify the cultures is shown in table 4.

There was some variation in the yields of acetylmethylcarbinol plus diacetyl with the different gases and a very definite variation in the yields of diacetyl. The largest yield of diacetyl was obtained with oxygen and the next largest yield with exposure to air, while carbon dioxide, hydrogen and nitrogen did not give appreciable quantities of diacetyl. The relatively high yield of diacetyl when oxygen was bubbled through the culture accompanied a comparatively low yield of acetylmethylcarbinol plus diacetyl.

When acetylmethylcarbinol is added to an unacidified tomato

TABLE 4. EFFECT OF VARIOUS GASES ON A CULTURE OF ONE OF THE CITRIC ACID-FERMENTING STREPTOCOCCI ACIDIFIED WITH CITRIC ACID.

Sterile skimmilk inoculated and held 18 hours at 21°C.; 0.7% citric acid added; various gases bubbled through different portions and containers sealed; amc +ac_2 and ac_2 determined after holding 48 hours at 21°C.

Organism used	pH after adding acid	Gas bubbled through milk	gm. Ni dimethylglyoximate ⥂ to	
			amc +ac_2 per 200 gm.	ac_2 per 200 gm.
32	3.7	none	0.0722	0.0020
		CO_2	.0813	none
		H_2	.0827	none
		N_2	.0772	none
		O_2	.0605	.0059
		O_2	.0641	.0057

bouillon or milk culture of one of the citric acid-fermenting organisms, there is a rapid reduction to 2,3-butylene glycol and, if diacetyl is added, there is a reduction to the glycol or to the carbinol (1). This general relationship makes it improbable that added acetylmethylcarbinol would be oxidized to diacetyl by the organisms in an unacidified culture but a few trials were carried out in an attempt to find small quantities of diacetyl in a culture in which the main change was a reduction of the carbinol. Both tomato bouillon and milk were used with carbon dioxide, hydrogen, nitrogen, oxygen and without added gas but appreciable quantities of diacetyl were never detected. The acetylmethylcarbinol largely disappeared during the holding period. Trials were also carried out with tomato bouillon, using cultures that had been acidified with sulfuric acid to a pH of about 3.9 in order to delay the reduction by the organisms, but appreciable quantities of diacetyl were not found; comparable trials with milk were not used because the presence of citric acid in the milk would have resulted in a production of acetylmethylcarbinol plus diacetyl at a low pH.

The results obtained with pure cultures of the citric acid-fermenting organisms are not directly applicable to butter cultures because (a) the typical lactic acid-producing organisms in a butter culture may influence the changes that occur and (b) there are smaller numbers of the citric acid-fermenting organisms per milliliter in a butter culture than in the pure cultures used. The action of various gases on butter cultures was studied as follows: Pasteurized skimmilk was inoculated with a butter culture and 0.15 percent citric acid added. Various gases were then bubbled through different portions, the cultures held at 21° C. for 15 to 20 hours and the determinations made after holding the ripened cultures in a refrigerator for 19 to 24 hours. Table 5 gives the results obtained.

TABLE 5. EFFECT OF VARIOUS GASES ON BUTTER CULTURES.

Pasteurized skimmilk inoculated and 0.15% citric acid added; various gases bubbled through different portions and containers sealed; held from 15 to 20 hours at 21°C.; removed to refrigerator and amc+ac_2 and ac_2 determined after 19 to 24 hours.

Butter culture used	Gas bubbled through milk	pH on analysis	gm. Ni dimethylglyoxime ⪯ to	
			amc+ac_2 per 200 gm.	ac_2 per 400 gm.
H5	none	4.4	0.0747	none
	CO_2	4.4	.0473	none
	H_2	4.5	.0797	none
	N_2	4.4	.0390	none
	O_2	4.5	.0650	0.00095
	O_2	4.5	.0654	.00125
122-F	none	4.4	.0858	none
	CO_2	4.5	.0686	none
	H_2	4.5	.0876	none
	N_2	4.4	.0892	none
	O_2	4.5	.0882	.00295
122-F	none	4.4	.0843	.00695
	CO_2	4.4	.0694	.0045
	H_2	4.5	.0884	.00635
	O_2	4.5	.0900	.0095
146	none	4.3	.0628	.0022
	CO_2	4.4	.0485	.0013
	CO_2	4.4	.0499	.0011
	H_2	4.7	.0638	.0018
	N_2	4.3	.0654	.0021
	O_2	4.4	.0609	.00335
	O_2	4.4	.0569	.0030
122-15	none	4.4	.0656	none
	O_2	4.5	.0635	.0023
232	none	4.4	.1141	none
	CO_2	4.4	.0851	none
	O_2	4.5	.1073	.0034
M1	none	4.3	.0569	none
	O_2	4.4	.0642	.00085

The variations in the yields of acetylmethylcarbinol plus diacetyl with the various gases were rather large, particularly in certain of the trials. There were also variations in the yields of diacetyl, with the portions through which oxygen had been bubbled regularly showing the highest yields. The portions with which carbon dioxide, hydrogen, nitrogen or air had been used frequently did not show appreciable quantities of diacetyl but, when they did, air gave higher yields than carbon dioxide, hydrogen or nitrogen. The variations in the yields of diacetyl in the different trials are rather striking. They cannot be attributed to differences in the butter cultures employed since one of the trials with 122-F gave appreciable quantities of diacetyl only when oxygen was used while the other gave appreciable quantities under all the conditions and, with oxygen, yielded much more diacetyl than in the other trial.

The action of oxygen on butter cultures made with various amounts of citric acid was studied by inoculating pasteurized skimmilk, dividing it, adding different amounts of citric acid to different portions and bubbling oxygen through some portions; after incubation the cultures were removed to a refrigerator and the usual determinations made after 24 hours. The data obtained are given in table 6.

TABLE 6. EFFECT OF OXYGEN ON BUTTER CULTURE MADE WITH VARIOUS AMOUNTS OF CITRIC ACID ADDED TO THE MILK.

Pasteurized skimmilk inoculated with culture 122; divided and various amounts of citric acid added to different portions; oxygen bubbled through certain portions and containers sealed; held 20 hours at 21°C.; removed to refrigerator and amc+ac_2 determined after 24 hours.

Citric acid added	No gas bubbled through		Oxygen bubbled through	
	gm. Ni dimethylglyoximate ≎ to		gm. Ni dimethylglyoximate ≎ to	
	amc+ac_2 per 200 gm.	ac_2 per 400 gm.	amc+ac_2 per 200 gm.	ac_2 per 400 gm.
none	0.0414	none	0.0354	none
0.1%	.0699	none	.0629	0.0017
.2	.0918	none	.0831	.0030
.3	.1015	none	.1139	.00455

Either with or without oxygen, the addition of the increasing amounts of citric acid gave a progressive increase in the yields of acetylmethylcarbinol plus diacetyl. Without oxygen no appreciable production of diacetyl occurred while with oxygen appreciable quantities were obtained in all cases, except the control to which no citric acid had been added, and the yields of diacetyl increased as the yields of acetylmethylcarbinol increased.

The influence of the addition of citric acid to the milk intended for butter culture when oxygen was bubbled through the culture was studied by inoculating pasteurized skimmilk and adding citric acid to some portions; after incubating 5 hours at 21° C. citric acid was added to the remaining portions and oxygen bubbled through all the lots; following additional incubation the cultures were removed to the refrigerator and subjected to the usual analyses after 6 hours and again after 24 hours. The butter culture used was one which appeared to be especially active in the production of diacetyl. Table 7 presents the data obtained.

The addition of citric acid regularly gave an increase in the yields of acetylmethylcarbinol plus diacetyl that was usually roughly proportional to the amounts of citric acid used. With either 0.2 or 0.3 percent citric acid the addition of the acid at the time of inoculation gave a larger yield of acetylmethyl-

TABLE 7. EFFECT OF VARIOUS AMOUNTS OF CITRIC ACID ON A BUTTER CULTURE WHEN OXYGEN WAS BUBBLED THROUGH.

Pasteurized skimmilk inoculated with butter culture 232; various amounts of citric acid added to some portions at time of inoculation and to others 5 hours later; oxygen bubbled through all portions 5 hours after inoculation; incubated 21 hours at 21°C.; removed to refrigerator and amc + ac_2 and ac_2 determined after 6 hours and again after 24 hours.

Citric acid added		1st analysis		2nd analysis	
		gm. Ni dimethylglyoxime ⇌ to		gm. Ni dimethylglyoxime ⇌ to	
At time of inoc.	5 hours after inoc.	amc + ac_2 per 200 gm.	ac_2 per 400 gm.	amc + ac_2 per 200 gm.	ac_2 per 400 gm.
none	none	0.0342	0.00235	0.0524	0.00335
0.1%		.0646	.00345	.0870	.0058
.2		.0954	.0038	.1144	.00685
.3		.1360	.0068	.1548	.00985
	0.1%	.0646	.0024	.0894	.00485
	.2	.0798	.0035	.1050	.00635
	.3	.0746	.0071	.1062	.0094

carbinol plus diacetyl than the addition 5 hours after inoculation. Accompanying the increased production of acetylmethylcarbinol plus diacetyl there was an increase in the production of diacetyl that was especially striking in the comparison of the effects of 0.2 and 0.3 percent citric acid. The results also show the influence of holding a ripened butter culture in the refrigerator for some time. In all cases, more acetylmethylcarbinol plus diacetyl was found at the second analysis than at the first and as a rule the difference was striking; the yield of the control culture at the second analysis was unusually high for a culture made without added citric acid. The increased production of acetylmethylcarbinol plus diacetyl on extended holding in the refrigerator was regularly accompanied by an increase in the diacetyl content.

Because of the possibility that *Streptococcus lactis* is involved in the oxidation of acetylmethylcarbinol in a butter culture, a number of trials were carried out with this species. The procedure used was to add the carbinol to sterilized milk, inoculate with *S. lactis* and then divide the milk into portions and pass carbon dioxide through one portion and oxygen through another; a control, which was not treated with gas, was also employed. In addition, each *S. lactis* culture was grown in milk to which no carbinol had been added. Diacetyl and acetylmethylcarbinol plus diacetyl were determined at the time of inoculation and again after 24 hours at 21° C. (when coagulation was complete) plus 24 hours in the refrigerator. There was no evidence of an oxidation of the carbinol to diacetyl in any of the trials. With one of the *S. lactis* cultures, a trace of diacetyl was produced in milk alone.

DISCUSSION OF RESULTS

The results presented indicate that the formation of diacetyl from acetylmethylcarbinol in a pure culture of a citric acid-fermenting Streptococcus, or in a butter culture, is due to the activity of organisms rather than to a simple chemical oxidation. In the case of a butter culture, *S. lactis* apparently is of no importance in this connection. Although accurate comparisons cannot be made, there appears to have been a relatively larger production of diacetyl in pure cultures of the citric acid-fermenting streptococci than in butter cultures. This may have been due to the comparatively small number of citric acid fermenters in the butter cultures, but the presence of *S. lactis* is a possible factor also. It should be noted that, because of the difficulties involved in the determination of extremely small amounts of diacetyl, the butter cultures in which appreciable quantities of diacetyl were not found may have contained enough of this compound to definitely influence the odor.

While the results obtained cannot be applied directly to butter, they suggest that in this product acetylmethylcarbinol is oxidized to diacetyl through the activity of organisms. This general relationship is in agreement with the many observations on the comparative flavor development in unsalted and normally salted butter. With unsalted butter, in which the butter culture organisms can multiply actively (4), a conspicuous increase in the volume of the desirable flavor is frequently noted when the butter is held at a temperature that is at all favorable for the growth of butter culture organisms, while with normally salted butter, in which the culture organisms fail to multiply, there is usually no significant increase in the desirable flavor. In butter with a relatively low salt content the culture organisms would be expected to multiply to some extent and such butter may show a definite increase in the volume of the desirable flavor.

If the action of butter culture organisms is necessary for the oxidation of acetylmethylcarbinol to diacetyl in butter, the presence of the carbinol in unsalted butter would be of questionable value, from the standpoint of flavor development, unless butter culture were used in the manufacture, and the same is true of its presence in butter containing culture together with sufficient salt to prevent the development of the culture organisms. Acetylmethylcarbinol may be present in butter made without culture as a result of bacterial action in the cream or the actual addition to either cream or butter. It appears that when conditions are unsatisfactory for the growth of the butter culture organisms in butter, the oxidation of acetylmethylcarbinol to diacetyl must occur in the butter culture or in the cream.

The conspicuous odor that is often noted in commercial preparations of acetylmethylcarbinol is commonly regarded as due to diacetyl formed through an oxidation of the carbinol. Diacetyl can be detected readily in such preparations by the usual analytical procedures. The formation of diacetyl in preparations of acetylmethylcarbinol without the action of organisms is not necessarily at variance with the idea that organisms are required for such an oxidation in a butter culture. The oxygen of the air may be more effective in oxidizing acetylmethylcarbinol in a concentrated form, especially in the absence of the complex compounds found in milk. However, it is difficult to understand how extensive oxidation could occur with the limited oxygen supply available in some of the containers used for preparations of acetylmethylcarbinol. A rearrangement of two molecules of the carbinol so as to yield one molecule of diacetyl and one of 2,3-butylene glycol is another possible source of diacetyl. It should be noted also that some preparations of purified acetylmethylcarbinol remain free from the odor of diacetyl for extended periods of time even with an abundant air supply.

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